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A Novel Plant Cell Culture Platform for Semicontinuous Production of Recombinant Proteins: Butyrylcholinesterase as a Case Study

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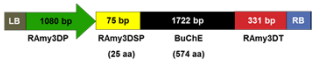
Abstract

In this poster we describe a novel biomanufacturing production platform that utilizes transgenic rice cell suspension cultures for efficient semicontinuous cell culture production of recombinant proteins. This platform offers a number of advantages over traditional methods for production of recombinant therapeutic proteins that use *E. coli*, yeast or mammalian cell cultures, while still retaining the ability to meet cGMP regulatory requirements under well-controlled, reproducible production conditions. Results are presented for semicontinuous production of butyrylcholinesterase, a bioscavenger for organophosphorus nerve agents such as sarin, using metabolically regulated transgenic rice cell cultures in a 5 L bioreactor.

Materials and Methods



	Native BuChE	Rice-Optimized BuChE
% base pairs different	25.3%	
% codons different	64.1%	
Codon Adaptation Index	0.68	0.83
Nc (effective # of codons)	47	41
Overall GC Content (%)	40.2	51.4
%GC in 1st place	46.2	49.1
%GC in 2nd place	40.2	40.2
%GC in 3rd place	34.3	64.9

Figure 2: Schematic of the gene construct and codon optimization data.

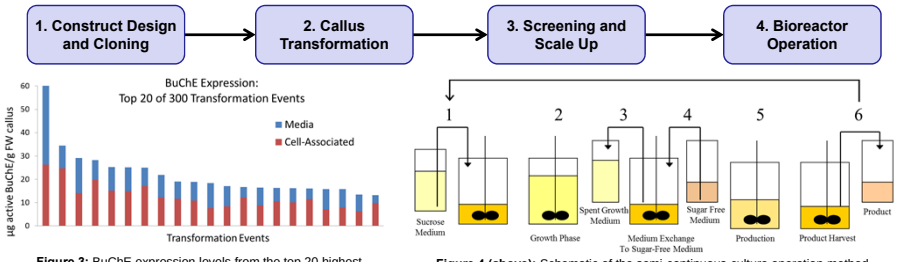


Figure 3: BuChE expression levels from the top 20 highest expressing transformation events of 300 total events screened.

Figure 4 (above): Schematic of the semi-continuous culture operation method.
Figure 5 (right): Transgenic rice cell culture grown in a 5 L bioreactor.

Background

Traditional methods for production of biologics use genetically modified *E. coli*, yeast, insect or mammalian cell cultures in bioreactor systems. For applications where a human therapeutic protein (monoclonal antibodies, vaccines, bioscavengers, replacement biologics) produced under strict cGMP conditions is required, plant cell cultures offer a number of advantages over currently used bioreactor-based systems:

- Low risk of contamination by mammalian viruses, blood-borne pathogens, prions or bacterial endotoxins or mycoplasma
- Ability to perform complex glycosylation
- Ease of culturing compared with other higher eukaryotic hosts
- Ability to grow in simple, low cost, chemically defined and animal component-free medium
- Established regulatory pathway for plant-based recombinant biologics for use as a human therapeutic

In the presented work, we focus on butyrylcholinesterase (BuChE) as a case study. BuChE is a tetrameric human enzyme that can act as a bioscavenger against organophosphorus nerve agents (Figure 1). Because of the high cost and limited availability of BuChE from human blood plasma, there is a need for a low cost, scalable recombinant platform for BuChE production.

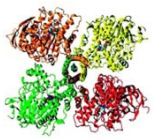
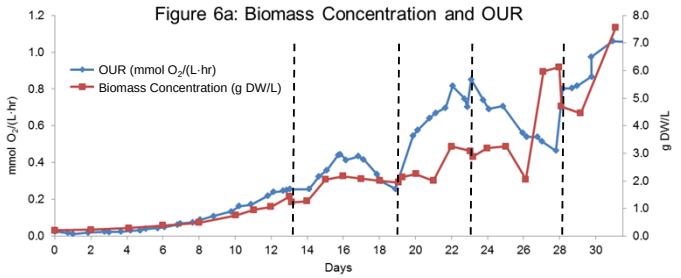


Figure 1: The tetrameric form of BuChE is 340 kDa[1].

Expression of the BuChE gene is controlled by the rice alpha amylase 3D promoter[2], which is activated by switching the rice from a sucrose-rich growth medium to a sucrose-free induction medium. This allows for a cyclical or semi-continuous culture operation that alternates between phases of cell growth (sugar-rich) and expression (sugar-free). Gravity sedimentation within the bioreactor can be used to separate the plant cell aggregates from the liquid phase, and the product collected can be purified either using a batch downstream strategy or fed to a continuous downstream process.

Results: Semicontinuous Bioreactor Operation



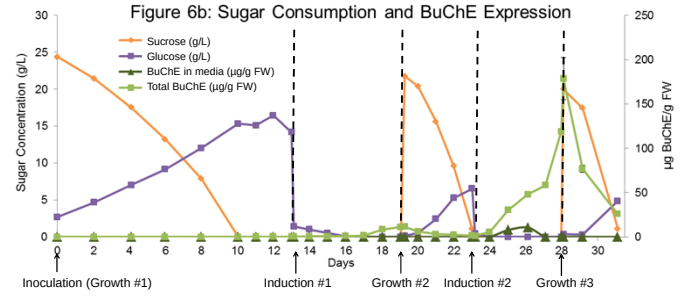


Figure 6: Kinetic data from semicontinuous production of BuChE in transgenic rice cell cultures under controlled conditions of temperature (27°C), agitation (75 rpm), and dissolved oxygen (40% of saturation with air). in a 5 L bioreactor.

Figure 6a: Cell growth and oxygen uptake rate (OUR) kinetics.

Figure 6b: BuChE production and sugar consumption kinetics. Active BuChE concentration is determined by a modified Ellman activity assay[3].

	Growth Phase #1	Growth Phase #2
Maximum Specific Growth Rate (μ_{max})	0.17 ± 0.01 day^{-1}	0.16 ± 0.02 day^{-1}
Doubling Time (τ_D)	4.0 ± 0.2 days	4.4 ± 0.7 days

	Expression Phase #1	Expression Phase #2
Active BuChE Yield	11.5 ± 0.1 μg/g FW	178.4 ± 23.6 μg/g FW
	0.306 ± 0.003 mg/L culture	8.4 ± 1.1 mg/L culture
Max Volumetric Productivity	16.1 ± 0.1 μg/(L·day)	954.8 ± 0.1 μg/(L·day)

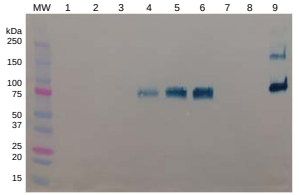


Figure 7a	
#	Contents
1	Day 0—immediately before Induction #1
2	Day 0—immediately after Induction #1
3	Day 2 of Induction #1
4	Day 4 of Induction #1
5	Day 5—immediately before Growth #2
6	Day 5—immediately after Growth #2
7	Day 7 of Growth #2
8	Day 9 of Growth #2
9	Equine BuChE Control (900 ng)

Figure 7: Western blotting of rice cell culture-produced BuChE.

Figure 7a: Western blot under reducing conditions of cell-associated samples from before and after medium exchanges. Each lane contains 20 μL of crude cell extract, obtained by grinding cells 1:1 in cold extraction buffer using a tissue homogenizer. Maximum BuChE expression occurs on day 5 after induction.

Figure 7b: Western blot under native conditions of 52 mU (~200ng) active BuChE from an intracellular sample from the first bioreactor expression phase (Induction #1). Tetrameric BuChE (~340 kDa) is observed.

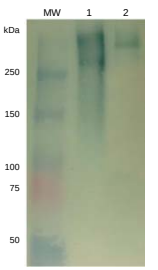


Figure 7b	
#	Contents
1	Induction #1
2	Equine BuChE Control

Conclusions

- Confirmed multiple stable transgenic cell lines capable of producing BuChE in a suspension culture.
- Successfully produced mg quantities of functional BuChE semi-continuously in a lab scale bioreactor.
- Induction #2 shows active BuChE present in the media at levels comparable to active cell-associated BuChE in Induction #1.
- Preliminary data indicate the production of tetrameric BuChE.

Future Work

- Optimization of bioreactor operating parameters, such as inoculation density, aggregate size distribution, dissolved oxygen, temperature, pH, and timing of induction in order to maximize culture health and volumetric productivity of functional BuChE.
- Evaluation of long-term (> 1 month) semicontinuous BuChE production.
- Purification and biochemical characterization of rice cell culture-produced BuChE and scale up of process.

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